Clinical Wound Healing Using Signal Inhibitors

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Abstract The cases presented in this chapter demonstrate the amazing improvement in wound-healing trajectory that is best explained by the suppression of biofilms. The individual patient with a chronic wound is not much better served today than a decade ago despite all of the advances achieved. The rate of major limb amputation and mortality is not decreasing, and the quality of life of these patients is not improving. Chronic wounds have a much higher prevalence rate of biofilms than acute ones, suggesting that presence of a biofilm is an important factor in the lack of healing of chronic wounds. As shown in this chapter, the implementation of the quorum-sensing inhibitor RNAIII-inhibiting peptide in managing complex wounds, along with other anti-biofilm strategies like lactoferrin, has dramatically changed the positive outcomes for many of the most desperate wounds.

1 Biofilm and Chronic Wound Infection

There has been an unprecedented technological explosion in science over the last decade, allowing for rapid expansion of our knowledge at the molecular and cellular levels of wound healing. Despite all our newfound knowledge, the general state of wound care is best summed up by Falanga, who stated, "Undoubtedly, we still have some way to go in the way we provide optimal care for our patients. A major stumbling block is the poor understanding of how chronic wounds fail to heal and what is their intrinsic pathogenesis that needs to be corrected" (Falanga 2001). Chronic wounds, and indeed most chronic human infections, continue to evade solid scientific explanation.

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Chronic human infections, including chronic wounds, constitute up to 80% of all human infectious disease (Costerton et al. 1995). The cost of these chronic infections is a major portion of the healthcare budget and continues to grow at exponential rates (Smith 2004). Although the big picture of chronic infections is dismal, the devastation of this problem is best understood by looking at the individual patient.

The individual patient with a chronic wound is not much better served today than a decade ago despite all the advances in our understanding. The outcomes for a patient with a diabetic foot ulcer remain bleak. The rate of major limb amputation is not decreasing, the mortality associated with a major limb amputation is not declining, and the quality of life reported by these patients is not improving (Buzato et al. 2002; Peters et al. 2001). Based on the human suffering and mounting costs, innovative explanations and management of chronic human infections must be sought.

The dominant view of most human infections is based on a "planktonic" concept. For chronic wounds it is very common to see the bacterial burden on the surface of the wound explained as a "contamination-infection continuum" (Fig. 1).

This continuum suggests that bacteria randomly land on the surface of the wound, and if they can divide, they can then "colonize" the surface. These individual bacteria can then further divide, invading a short distance into the host. This is termed "critical colonization" and is identified clinically by local increased erythema, heat, pain, and swelling. If the individual bacteria can invade the host, broader involvement (infection) results. The only problem with the contamination-infection continuum is that there is no scientific validation for it, and it is a very minor pathway regarding how bacteria behave on a surface.

Endocarditis, prostatitis, sinusitis, otitis media, osteomyelitis, and other chronic biofilm infections all occur on surfaces. When a bacterium encounters a surface to which it can attach, it quickly forms a biofilm. A biofilm offers bacteria much more survivability, and therefore is the preferred phenotype in which bacteria choose to exist. Biofilm may be the best explanation for many of the clinical observations associated with chronic infections (Table 1).

As noted in other chapters, a biofilm is a colony of bacteria expressing different proteins (phenotypes) to fulfill the needs of the colony (Sauer et al. 2002). A biofilm should be considered a true multicellular organism with each individual under molecular control by the colony. The bacteria that constitute a biofilm usually arise from a number of bacterial species that work in synergy to ensure the colony's survival.

Chronic infections are very resistant to conventional treatments such as antibiotics because of the defenses that the biofilm colonies possess. Biofilms possess an extracellular polymeric substance (EPS) that protects the bacteria from environmental attacks (Costerton et al. 1999). Biofilms are also very resistant to biocides such as hydrogen peroxide, bleach, acetic acid, and others (Costerton and Stewart 2001). The biofilm phenotype, along with the physical barrier and structure provided by the EPS, makes biofilm almost impervious to host defenses (Stoodley et al. 2002). In fact, a strategy of suppression rather than eradication has yielded the best outcomes in chronic wounds (see below).

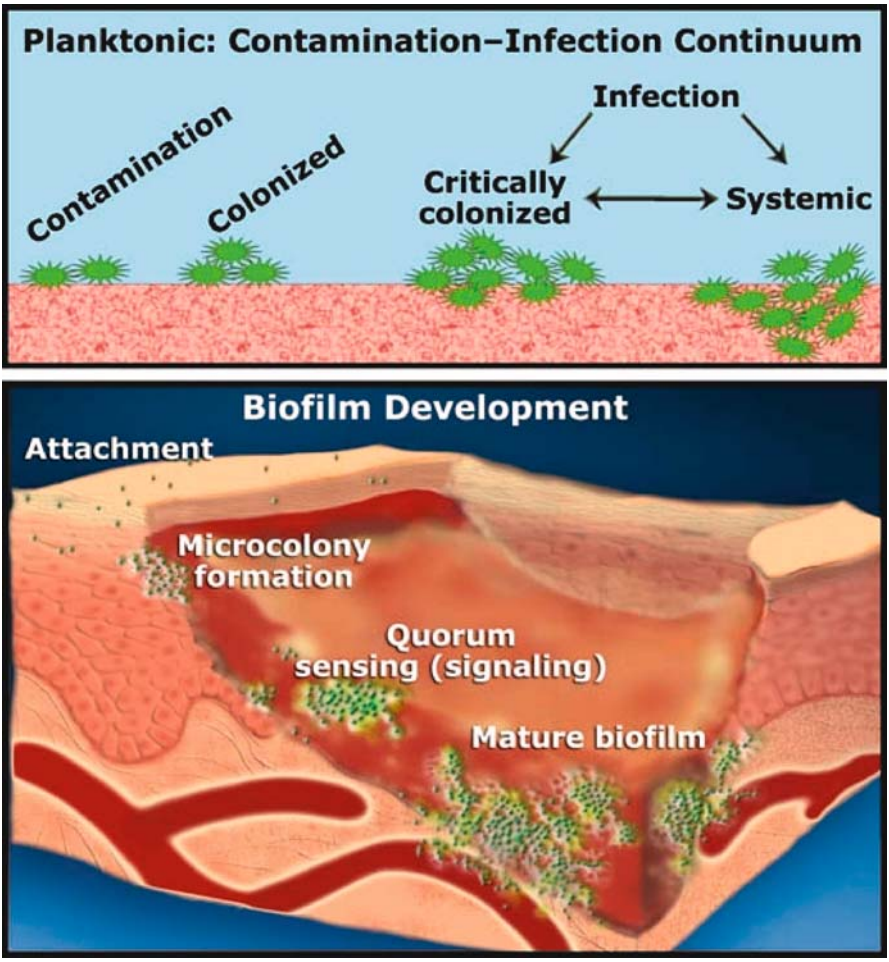

Fig. 1 A planktonic explanation for surface bacteria involved in wounds is not scientifically valid. Planktonic bacteria quickly form into microcolonies and then mature into formidable wound biofilm that evades host defenses and impairs wound healing

The process that may take place in the genesis of a chronic wound or other biofilm-based infection is as follows (Costerton et al. 1999): A planktonic bacterium finds its way through the first lines of defense, such as through a skin break, mucous membrane, or alveoli, and attaches to the underlying tissue (Fig. 2A). Once the bacterium attaches to exposed extracellular matrix components, it rapidly begins biofilm behavior to express new proteins and EPS. If the host immune system, mainly the innate system, fails to clear the bacteria before it is protected within its EPS fortress, then the immune system becomes irrelevant. Once the microcolony (early biofilm) is firmly entrenched on the tissue surface, it rapidly matures into a biofilm, which is protected from the host immune response and antibiotics (Fig. 2B,C).

Table 1 Observations in chronic wounds explained by the presence of a biofilm

Observation	Planktonic concept	Biofilm concept
In vitro	Planktonic bacterium (seed) expresses proteins and structures for motility and attachment (flagella, fimbria). Its function is to spread the colony to a different location.	Biofilm (vegetation) is a complex colony of bacteria that can express different proteins (phenotype) to fulfill different roles to help the community survive. Biofilm is stationary, protecting its location with multiple defenses.
In vivo	Planktonic bacteria are susceptible to antibiotics, biocides, and the immune system.	Biofilms are resistant to antibiotics and biocides. Once biofilm is established, it cannot be eradicated by the immune system.
Acute wounds heal in 2–4 weeks; chronic wounds in the same area heal in 4–6 months	Acute wounds have intact defenses and clear planktonic bacteria not allowing biofilm formation.	Random planktonic bacteria evade host defenses and set up biofilm. Biofilm possesses defenses against the host immune system and strategies to keep the wound open.
Antibiotics not effective in chronic wounds but work in acute wounds	Sensitive planktonic bacteria show a rapid 4- to 5-log reduction.	Sensitive biofilm phenotype bacteria show only a grudging 1- to 2-log reduction and quick adaptation.
Biocides not effective in chronic wounds	Nonspecific biocides eradicate planktonic bacteria.	Nonspecific biocides eradicate host healing cells and host defenses, doing little damage to the biofilm.
Drying the wound (open to air, heat lamp, etc.) is ineffective in chronic wounds	Planktonic bacteria are very sensitive to the environment.	Biofilms are very resistant to drying and other environmental changes.
Closing a traumatic wound after 4–12 h leads to increased dehiscence	Planktonic phenotypes dominate the surface early on (1–2 h) and are sensitive to biocides and amenable to closure.	Biofilms form in 1–2 h and are resistant to surgical scrubs. Closure of the wound puts two surfaces in contact with the biofilm and leads to dehiscence.
Increased surgical site infections in patients with chronic wounds	Surgical preps and prophylactic antibiotics are highly effective planktonic bacteria.	Chronic wounds shed biofilm fragments continuously. These fragments have all the defenses of biofilm and are resistant to surgical preps and prophylactic antibiotics.
Wet-to-dry dressings are detrimental to chronic wounds	Planktonic bacteria quickly (6 h) seed gauze and form biofilm on gauze.	Detachment fragments foul the gauze dressing, forming biofilm that produces fragments, toxins, etc., that are detrimental to the wound.

The biochemical impairment on the surface of a chronic wound is well documented. There are very high levels of MMP8, which is the major matrix metalloprotease of neutrophils. Yeager and Nwomeh have stated, “Virtually all the available

Table 1 (continued)

Observation	Planktonic concept	Biofilm concept
Autograft or allograft failure on wounds	Planktonic cells are easily cleared by neutrophils, antibodies, and common wound-bed preparations.	Laying unprotected cells over biofilm just adds a second surface and a food source, leading to the rapid deterioration of the graft, with increased exudate, inflammation, and odor.
Negative wound cultures	Most clinical bacteria in planktonic phenotype are easily cultured.	Wounds have bacteria on their surface yet culture negative when only biofilm phenotype (i.e., viable but not culturable) is present.
Wounds "stuck" in chronic inflammatory state	Planktonic bacteria are easily cleared by host's inflammatory response and try to avoid causing inflammation.	Biofilms are impervious to the host's inflammatory response and can even feed off the exudate produced by inflammation. Biofilms try to cause inflammation.
Corticosteroids known to slow the host healing response help heal some chronic wounds	Steroids decrease the host immune response, making planktonic infections worse.	Steroids rob biofilms of nutrition by decreasing the host inflammatory response.
No blood clot formation on chronic wound	Planktonic bacteria are overwhelmed by host defenses after injury, and clot will form on wound surface.	Biofilms prevent clot formation on the surface of a wound post-debridement by direct competition and an increased proteolytic environment.

evidence supports a role for neutrophils in the pathophysiology of chronic wounds" (Yager and Nwomeh 1999). Diegelman concurred, stating, "In contrast, the ulcer microenvironment appears to be an area of massive destruction due to the invasion of tremendous numbers of neutrophils and their destructive enzymes" (Diegelmann 2003).

There have been tremendous advances in imaging over the last decade, yet very few studies have examined the wound bed itself. Diegelman's study looking at tissue gram stains to demonstrate the presence of excessive neutrophils inadvertently demonstrated the presence of wound biofilm in the same area (Diegelmann 2003). The presence of wound biofilm can explain the elevated proinflammatory cytokines, elevated matrix metalloproteases, and excessive neutrophils seen in all chronic wounds.

There is now good evidence that chronic wounds are different from acute wounds because the former possess biofilm on their surface. Electron microscopy studies along with tissue gram stains conducted at the Center for Biofilm Engineering found that chronic wounds had a high prevalence of biofilm on the surface of the wound (Fig. 3a), whereas acute wounds rarely exhibited any biofilm (Fig. 3b) (James et al., unpublished). This would indicate that the difference between an acute and a chronic wound is the presence of biofilm on the chronic wound (Fig. 4).

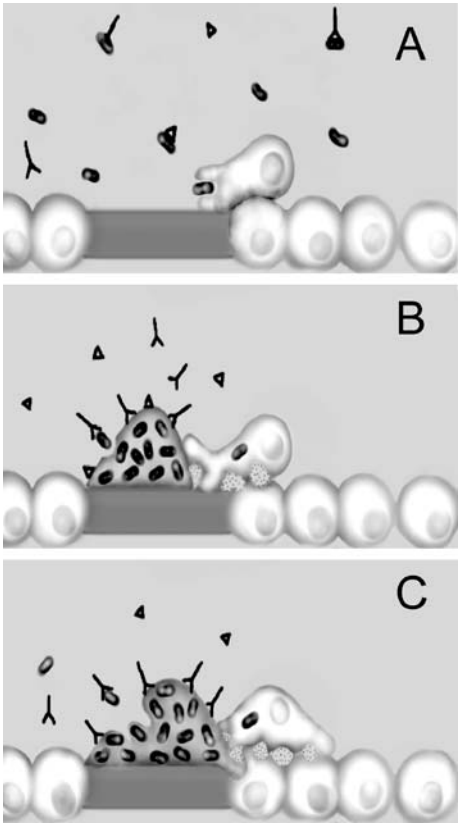

Fig. 2 Diagram of implant infection. **A** Planktonic bacteria are susceptible to the host's immune response and to antibiotics. **B** Bacteria form biofilms, such as on medical devices, where they are resistant to immune response and to antibiotics. **C** Phagocytes attach to the biofilm. They are unable to phagocytose the bacteria, but degrading enzymes are released, destroying surrounding tissue. Some bacteria are released, leading to dissemination of infection to neighboring tissue. (*Y* antibodies, Δ-antibiotics) (Illustration by Mike Beshiri, Tufts University, Cummings School of Veterinary Medicine, Department of Biomedical Sciences, Division of Infectious Diseases, North Grafton, MA, USA)

Chronic wounds, and indeed all biofilm-based human infections, are locked in a persistent chronic inflammatory state in a high proteolytic environment. As noted above, chronic wounds seem to have significant biofilm, whereas acute wounds (which do not exhibit a chronic inflammatory state) have very little biofilm on their surface. Intuitively, it makes sense for the presence of a colony of bacteria to produce a vigorous immune response from the host. But is it biofilm on the wound's surface that is causing the chronic inflammation and thus impairing healing? If wound healing improves with the use of agents that only target the biofilm behavior of bacteria, then the biofilm must be contributing to the impairment in healing. This provides indirect evidence of the role of biofilm in nonhealing chronic wounds.

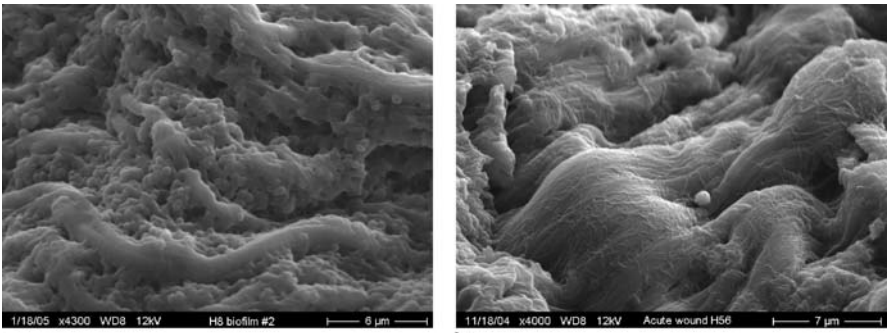

Fig. 3 a Wound biofilm as seen in this electron micrograph covers most of the surface of chronic wounds regardless of the etiology. **b** Acute wounds show expanses of extracellular matrix with occasional planktonic bacteria. Acute wounds have little biofilm on their surface

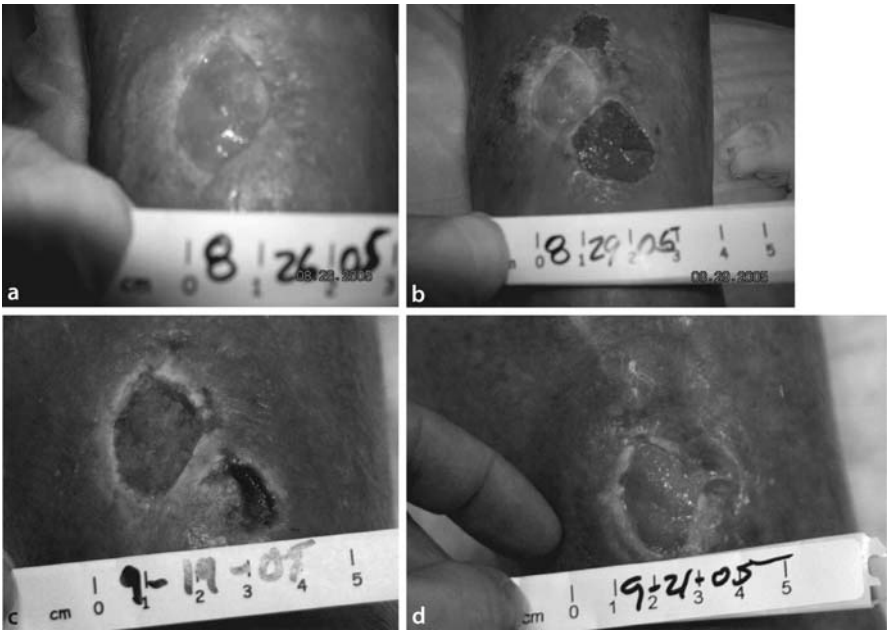

Fig. 4 This series of photographs captures the development of a full-thickness acute wound in the same region as a chronic wound. The acute wound healed in 3 weeks, but the chronic wound did not. The difference may be biofilm

Quorum-sensing inhibitors (QSIs) have the unique property of affecting only the biofilm behavior of bacteria. As noted in other chapters, quorum-sensing molecules are specific signaling agents that control the behavior of biofilms by regulating the gene expression of each of the colony bacteria. The original name given to these signaling molecules was quorum-sensing molecules because a critical density of individual bacteria (a quorum) is required to trigger biofilm gene expression. In other

words, a certain number of these molecules need to be present to cause upregulation or downregulation of suites of genes (operons and regulons) responsible for biofilm formation. It was clear from the beginning that if this communication language could be understood, certain behaviors could be manipulated (inhibited), thus averting the impenetrable defenses of biofilm.

As noted in other chapters, RIP is a staphylococcal QSI that blocks biofilm behavior and toxin production. Animal studies using rat graft or central venous catheter models show that animals treated with RIP were able to clear *S. aureus* and *S. epidermidis* biofilms and that RIP is synergistic with commonly used antibiotics. RIP suppressed any type of staphylococcal infection, including drug-resistant ones such as methicillin-resistant *S. aureus* (MRSA), methicillin-resistant *S. epidermidis*, vancomycin-intermediate *S. aureus*, and vancomycin-intermediate *S. epidermidis* (e.g., Balaban et al. 2005). This is a very exciting step toward specific, well-understood agents that may be useful in a clinical setting.

2 Use of a Quorum-Sensing Inhibitor to Treat Chronic Wound Infections

Two cases demonstrate the potential power of a QSI (RIP) in clinical application for controlling biofilm-based infectious diseases. RIP was used in these two cases of chronic wounds with osteomyelitis in patients who had exhausted all other options. Although the methods of using RIP are still in development, these two cases demonstrate the potential of QSIs for future clinical use.

2.1 Case History 1

A man with severe peripheral vascular disease and diabetes mellitus sustained minor trauma to his right little toe. In his minor diabetic foot ulcer he was found to have MRSA, *Enterobacter cloacae*, *Pseudomonas* species, *Citrobacter* species, *E. coli*, and group D enterococci. Over the next 6 months, the patient had aggressive conservative wound care. This included topical products such as Iodosorb, Hydrofera Blue, Acticoat, silver gels, enzymes, Promogran, and many other topical preparations. The wound underwent debridement frequently. Despite these measures, there was a continual dying back into the forefoot. Perfusion and oxygenation studies showed that there was adequate blood flow and oxygenation of the tissues for healing. Yet the wound relentlessly worsened. There was extensive osteomyelitis across the forefoot within 4 months. The patient was offered major limb amputation on several occasions but refused. After 7 months (Fig. 5a), the patient had just completed 2 weeks of intravenous Cubicin at 6 mg/kg along with topical application of Hydrofera Blue on a daily basis, offloading, and nutritional support. The foot was clearly worsening. After 8 months, a gel preparation of RIP at 1.7 mcg/ml was ap-

plied topically to the wound on a daily basis along with continued use of Cubicin 4 mg/kg for 3 weeks. By that time, the wound had shown significant improvement. The Cubicin was stopped, but the RIP gel was continued with steady improvement noted throughout the next few months (Fig. 5b). Three months after that, Apligraf grafting was instituted every 2–4 weeks for the next 8 months to completed healing (Fig. 5c). In summary, RIP, as a topically applied agent used adjunctively with intravenous antibiotics, cleared the biofilm that was slowly eroding through the foot. This treatment produced a spectacular salvage of this patient's limb.

2.2 Case History 2

A man with severe insulin-dependent diabetes mellitus presented with a hot, swollen left foot with blood sugars greater than 600. He was febrile and hypotensive and was immediately admitted to the hospital for intravenous antibiotics and surgical management of his left foot. The surgical decision was made to amputate the left lower extremity, which the patient refused. Local surgery was done on the foot, which had significant pus in the forefoot with osteomyelitis involving the tarsal and metatarsals. The organisms cultured were MRSA and group D enterococci. The patient was placed on Cubicin 6 mg/kg and continued to do poorly (Fig. 6a). He was dismissed from orthopedic and medical care because he continued to refuse amputation of his left lower extremity. He was placed in outpatient therapy with daily Cubicin 6 mg/kg and was injected with RIP in the tarsal area (25 mg/5 ml Marcaine). This injection was done on a daily basis for three visits. The local wound care consisted of daily ultrasonic debridement followed by sharp debridement to open up any undermining and tunneling and to remove any necrotic material. Applied topical preparations included lactoferrin 10 mg/ml, xylitol 5% by weight, and RIP 1.7 μg/ml. The compounded gel was placed on the foot on a daily basis. After 1 week, the patient's blood sugars had normalized, his malaise had cleared, and the wound was much improved. The foot edema and erythema completely resolved within 10 days (Fig. 6b). The injection of RIP in the area of obvious *S. aureus* biofilm (osteomyelitis) markedly changed the patient's clinical course and led to resolution of the osteomyelitis as well as healing of the diabetic foot ulcer (Fig. 6c).

QSIs are not the only signal-inhibiting agents that have been shown to improve wound healing by suppressing biofilm. Lactoferrin applied topically to a chronic wound has shown good ability to block reaccumulation of biofilm after debridement (see case history 3, below). Xylitol and other alcohol sugars have demonstrated an ability to weaken EPS, and therefore can be the first line of defense to improve wound outcomes. In fact, a number of excellent antibiofilm agents are well documented in the literature from other fields (Domenico et al. 2004; Katsuyama et al. 2005; Tapiainen et al. 2004; Veloira et al. 2003). The fact that these agents produce better outcomes in chronic biofilm infections demonstrates that the approach of direct biofilm suppression may be a valuable medical tool.

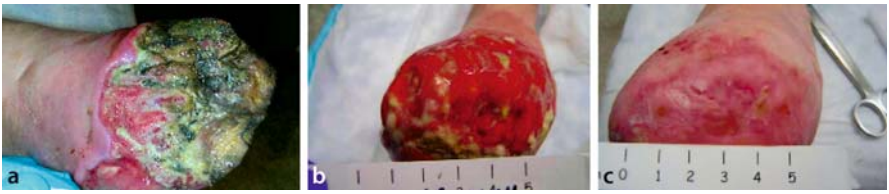

Fig. 5 Case history 1. The patient had continued dying back of the foot (**a**) secondary to infection caused by methicillin-resistant *S. aureus*, *Enterobacter cloacae*, *Pseudomonas* spp, *Citrobacter* species, *E. coli*, and group D enterococci. Once RIP was added to the regimen, there was a dramatic turnaround (**b**), and the patient went on to healing (**c**)

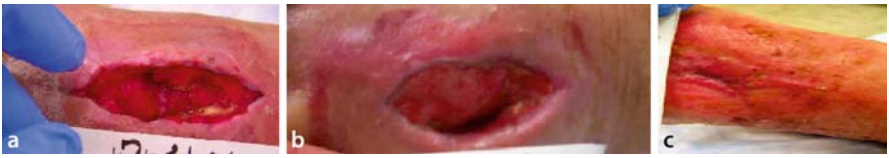

Fig. 6 Case history 2. The patient presented with overwhelming infection of the foot with osteomyelitis (**a**). The use of RIP injected in the tarsal and metatarsal region in conjunction with appropriate antibiotics (**b**) allowed for limb salvage (**c**)

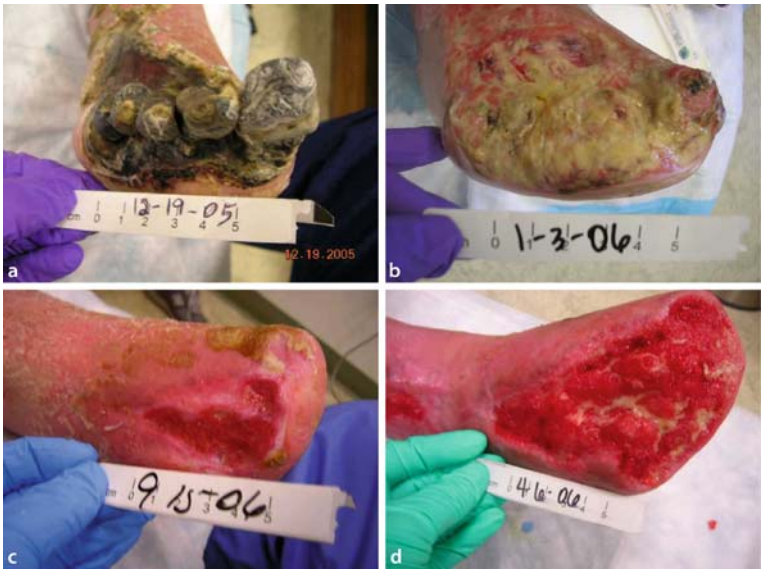

Fig. 7 Case history 3. The patient presented with overwhelming infection of the foot with osteomyelitis (**a**). The initial culture showed methicillin-resistant *S. aureus*, with subsequent culture showing *Enterococcus* and *E. coli*. Debridement was carried out at 1-week intervals. The patient was placed on Daptomycin 6 mg/kg for over 6 weeks (**b**). The topical regimen was lactoferrin 33 mg/cc, Xylitol 5%, and silver (Acticoat) (**c**), and later Apligraf and Oasis, allowing for limb salvage (**d**)

3 The Use of Lactoferrin in Wound Care

As noted in other chapters, lactoferrin is used in many products, as well as in meat packing plants, to treat meat surfaces to prevent biofilm formation and as a dietary supplement. It has been a popular nutritional supplement for a number of decades, a situation that may have contributed to a lack of rigorous study of this very powerful component of the innate immune system. Copious amounts of lactoferrin are secreted in human external secretions (tears, saliva, mucous, milk, etc.). Because lactoferrin is a main component of the innate immune system and is ubiquitous in surface secretions, it seems logical that lactoferrin would play a significant role in antimicrobial surface protection of the host. Exactly what roles it plays and how it plays these roles is just now coming to light.

The initial work of looking into the antimicrobial activity of lactoferrin suggested that the protein's affinity for iron (transferrin) was its main mechanism of action. It was demonstrated that lactoferrin sequesters iron, depleting this essential bacterial nutrient and causing a bacteriostatic action against bacteria (Bullen 1975). However, this research and several subsequent studies focused on planktonic bacteria, not biofilms (Weinberg 1993). It was only recently that the biofilm-lactoferrin connection was made (Weinberg 2004).

By 1977 there was some suggestion that the role of lactoferrin was more complex than just its ability to bind iron (Arnold et al. 1977). In planktonic bacteria, lactoferrin was found to have a direct bactericidal effect. Lactoferrin was found to bind to the lipopolysaccharide portion in the outer membrane of gram-negative bacteria. This binding to the phosphate group in the lipid A region of the membrane causes rigidification of acyl chains of the lipopolysaccharides, which causes rapid release of lipopolysaccharides and increased membrane permeability. It is increased membrane permeability that causes planktonic bacterial cell death (Brandenburg et al. 2001; Appelmelk et al. 1994; Ellison III et al. 1988).

Recently, a number of different functions have been identified for this versatile protein. For example, a definite protease activity has been identified. Lactoferrin acts as a serine protease that can cleave arginine-rich sequences. Independent from its iron binding capacity, it has been shown to induce degradation of bacterial secreted proteins necessary for attachment (Ochoa et al. 2003).

An important new finding is that lactoferrin works synergistically with polymorphonuclear cells to produce bactericidal activity. Lactoferrin acts as a reservoir for iron which is required for catalyzing hydroxyl radical production, one of the main weapons in the polymorphonuclear cells' armamentarium. It was found that lactoferrin remains stable and continues to bind iron even at a very low pH. The activated neutrophil binds lactoferrin-containing granules with the acidic phagolysosome, resulting in the needed iron source for its bactericidal activity (Ward et al. 2002).

Lactoferrin has been shown to prevent free-floating (planktonic) *P. aeruginosa* from attaching to a surface. If the attachment is prevented, then biofilm formation is prevented (Singh et al. 2002). It seems that as a planktonic bacterium adheres to a surface in the presence of lactoferrin, the movement of the individual bacteria (referred to as twitching) is increased. If twitching is increased for the parent cell

and all its progeny, they fail to group into microcolonies, which is the first step in expressing biofilm behavior. In that study, *P. aeruginosa* cultures without lactoferrin demonstrated thick biofilm formation. When lactoferrin was added, the planktonic bacteria were able to divide but not attach, so no biofilm structures were identified (Singh et al. 2002). Lactoferrin worked purely to prevent the attachment of planktonic bacteria, thus preventing biofilm formation.

To determine whether lactoferrin is effective in managing wounds, our group conducted an 8-week prospective randomized controlled study of 50 patients. Twenty-five patients with a total of 37 wounds were randomly placed in the lactoferrin gel treatment group. The control group consisted of 25 patients with 30 wounds, and they received the same gel without lactoferrin. The study demonstrated that patients receiving the lactoferrin gel had a higher percentage of complete healing in the 8 weeks. Overall, lactoferrin was an effective agent for improving the healing of chronic wounds (see case history 3).

3.1 Case History 3

A man had acute onset of necrosis of all the toes of his right foot, with critical limb ischemia. A stent was placed in the superficial femoral artery. The patient did reperfuse the foot but was still recommended to have a below-knee amputation (Fig. 7a). At the initial visit, the patient's $TcPO_2$ in the right foot was 23 mmHg. Over the course of treatment it rose slightly to 25 mmHg. Initial culture showed MRSA, with subsequent culture showing *Enterococcus* and *E. coli*. Biofilm-based wound management was initiated. Initially, debridement was targeted on removing all necrotic material and shaping the wound to benefit the host (Fig. 7b). Debridement was carried out at 1-week intervals. The patient was placed on daptomycin 6 mg/kg for over 6 weeks because he had osteomyelitis of the metatarsals; daptomycin has shown efficacy against biofilm phenotype bacteria. The topical regimen used was a mixture of lactoferrin 33 mg/cc, xylitol 5%, and Acticoat; lactoferrin, xylitol, and silver have shown good synergy in preventing reaccumulation of biofilm after removal by debridement (Fig. 7c). Surface management was continued on a weekly basis using ultrasonic debridement. By the 6th month, the biofilm was suppressed sufficiently to allow proactive agents such as Apligraf and Oasis to be applied to the wound to promote wound healing. These agents have worked effectively, and although the limb remained hypoxic, healing was almost complete by the 9th month (Fig. 7d).

Healing of a difficult, hypoxic wound in an elderly patient is difficult, and this case illustrates the relative contribution of wound biofilm in preventing wound healing. With wound biofilm suppressed, even difficult wounds demonstrate the ability to heal. It appears that wound biofilm is a major barrier to wound healing; therefore, specific targeting of wound biofilm should result in better wound healing outcomes.

4 Concluding Remarks

From a medical perspective, biofilm needs rigorous scientific attention. Wounds and other biofilm-based diseases account for a major portion of current healthcare expenditures. Biofilm diseases cause significant suffering, major disability, loss of limb, and loss of life. Molecular and cellular evaluation of biofilm and the host biofilm interaction is an important first step to mitigate this desperate situation.

The answer to biofilm infections will probably not be found in a single agent such as a single QSI. More likely, the answer will be found in a coordinated approach using multiple simultaneous strategies such as frequent repetitive removal of the biofilm, specific biocides, antibiofilm antibiotics (Cubicin) and multiple antibiofilm agents (Fux et al. 2005). Antibiofilm agents include QSIs that can act at different pathways to manipulate biofilm behavior. Using QSIs to attack biofilms simultaneously at attachment, differentiation, EPS production, and so on, may be a more robust approach. This gives much hope for eventually controlling biofilm to the point where it no longer produces devastating disease.

References

Appelmelk BJ, An YQ, Geerts M, Thijs BG, de Boer HA, MacLaren DM, de Graaff J, Nuijens JH (1994) Lactoferrin is a lipid A-binding protein. Infect Immun 62:2628–2632

Arnold RR, Cole MF, McGhee JR (1977) A bactericidal effect for human lactoferrin. Science 197:263–265

Balaban N, Stoodley P, Fux CA, Wilson S, Costerton JW, Dell'Acqua G (2005) Prevention of staphylococcal biofilm-associated infections by the quorum sensing inhibitor RIP. Clin Orthop Relat Res 437:48–54

Brandenburg K, Jurgens G, Muller M, Fukuoka S, Koch MH (2001) Biophysical characterization of lipopolysaccharide and lipid A inactivation by lactoferrin. Biol Chem 382:1215–1225

Bullen JJ (1975) Iron-binding proteins in milk and resistance to *Escherichia coli* infection in infants. Postgrad Med J 51(suppl 3):67–70

Buzato MA, Tribulatto EC, Costa SM, Zorn WG, van Bellen B (2002) Major amputations of the lower leg. The patients two years later. Acta Chir Belg 102:248–252

Costerton JW (2005) Biofilm theory can guide the treatment of device-related orthopaedic infections. Clin Orthop Relat Res 437:7–11

Costerton JW, Stewart PS (2001) Battling biofilms. Sci Am 285:74–81

Costerton JW, Stewart PS, Greenberg EP (1999) Bacterial biofilms: a common cause of persistent infections. Science 284:1318–1322

Diegelmann RF (2003) Excessive neutrophils characterize chronic pressure ulcers. Wound Repair Regen 11:490–495

Domenico P, Gurzenda E, Giacometti A, Cirioni O, Ghiselli R, Orlando F, Korem M, Saba V, Scalise G, Balaban N (2004) BisEDT and RIP act in synergy to prevent graft infections by resistant staphylococci. Peptides 25:2047–2053

Ellison RT III, Giehl TJ, LaForce FM (1988) Damage of the outer membrane of enteric gram-negative bacteria by lactoferrin and transferrin. Infect Immun 56:2774–2781

Falanga V (2001) Introduction, overview. In: Falanga V (ed) Cutaneous wound healing. Martin Dunitz, London, p 2

Fux CA, Costerton JW, Stewart PS, Stoodley P (2005) Survival strategies of infectious biofilms. Trends Microbiol 13:34–40

Katsuyama M, Kobayashi Y, Ichikawa H, Mizuno A, Miyachi Y, Matsunaga K, Kawashima M (2005) A novel method to control the balance of skin microflora. Part 2. A study to assess the effect of a cream containing farnesol and xylitol on atopic dry skin. J Dermatol Sci 38:207–213

Ochoa TJ, Noguera-Obenza M, Ebel F, Guzman CA, Gomez HF, Cleary TG (2003) Lactoferrin impairs type III secretory system function in enteropathogenic *Escherichia coli*. Infect Immun 71:5149–5155

Peters EJ, Childs MR, Wunderlich RP, Harkless LB, Armstrong DG, Lavery LA (2001) Functional status of persons with diabetes-related lower-extremity amputations. Diabetes Care 24:1799–1804

Sauer K, Camper AK, Ehrlich GD, Costerton JW, Davies DG (2002) *Pseudomonas aeruginosa* displays multiple phenotypes during development as a biofilm. J Bacteriol 184:1140–1154

Singh PK, Parsek MR, Greenberg EP, Welsh MJ (2002) A component of innate immunity prevents bacterial biofilm development. Nature 417:552–555

Smith A (2004) Etiology of the problem wound. In: Sheffield PJ, Fife CE, Smith A (eds) Wound care practice. Best, Flagstaff, pp 3–48

Stoodley P, Sauer K, Davies DG, Costerton JW (2002) Biofilms as complex differentiated communities. Annu Rev Microbiol 56:187–209

Tapiainen T, Sormunen R, Kaijalainen T, Kontiokari T, Ikaheimo I, Uhari M (2004) Ultrastructure of *Streptococcus pneumoniae* after exposure to xylitol. J Antimicrob Chemother 54:225–228

Veloira WG, Domenico P, LiPuma JJ, Davis JM, Gurzenda E, Kazzaz JA (2003) *In vitro* activity and synergy of bismuth thiols and tobramycin against *Burkholderia cepacia* complex. J Antimicrob Chemother 52:915–919

Ward PP, Uribe-Luna S, Conneely OM (2002) Lactoferrin and host defense. Biochem Cell Biol 80:95–102

Weinberg ED (1993) The development of awareness of iron-withholding defense. Perspect Biol Med 36:215–221

Weinberg ED (2004) Suppression of bacterial biofilm formation by iron limitation. Med Hypotheses 63:863–865

Yager DR, Nwomeh BC (1999) The proteolytic environment of chronic wounds. Wound Repair Regen 7:433–441